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Original article

A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant *Enterobacteriaceae*: a randomized clinical trial

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ABSTRACT

Objectives: Intestinal carriage with extended spectrum β-lactamase *Enterobacteriaceae* (ESBL-E) and carbapenemase-producing *Enterobacteriaceae* (CPE) can persist for months. We aimed to evaluate whether oral antibiotics followed by faecal microbiota transplantation (FMT) can eradicate intestinal carriage with ESBL-E/CPE.

Methods: Randomized, open-label, superiority trial in four tertiary-care centres (Geneva (G), Paris (P), Utrecht (U), Tel Aviv (T)). Non-immunocompromised adult patients were randomized 1: 1 to either no intervention (control) or a 5-day course of oral antibiotics (colistin sulphate 2×10^6 IU 4×/day; neomycin sulphate 500 mg 4×/day) followed by frozen FMT obtained from unrelated healthy donors. The primary outcome was detectable intestinal carriage of ESBL-E/CPE by stool culture 35–48 days after randomization (V4). ClinicalTrials.gov NCT02472600. The trial was funded by the European Commission (FP7).

Results: Thirty-nine patients (G = 14; P = 16; U = 7; T = 2) colonized by ESBL-E (n = 36) and/or CPE (n = 11) were enrolled between February 2016 and June 2017. In the intention-to-treat analysis 9/22 (41%) patients assigned to the intervention arm were negative for ESBL-E/CPE at V4 (1/22 not receiving the intervention imputed as positive) whereas in the control arm 5/17 (29%) patients were negative (one lost to follow up imputed as negative) resulting in an OR for decolonization success of 1.7 (95% CI 0.4–6.4). Study drugs were well tolerated overall but three patients in the intervention group prematurely stopped the study antibiotics because of diarrhoea (all received FMT).

Conclusions: Non-absorbable antibiotics followed by FMT slightly decreased ESBL-E/CPE carriage compared with controls; this difference was not statistically significant, potentially due to early trial

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termination. Further clinical investigations seem warranted. **B.D. Huttner, Clin Microbiol Infect 2019;25:830**

Introduction

Infections with extended-spectrum β-lactamase (ESBL-E) and carbapenemase (CPE) producing *Enterobacteriaceae* are associated with significant morbidity, costs and mortality [1–5]. Controlling the spread of ESBL-E and CPE is complicated by the persistence of intestinal carriage for months to years putting the carriers at risk for recurrent infections and representing a reservoir for transmission [6,7].

Several decolonization strategies for ESBL-E and CPE carriers have been examined but studies were of low methodological quality or only showed moderate efficacy [8]. A double-blind, placebo-controlled, randomized study conducted in Geneva in 2009–2012 examined the impact of oral neomycin and colistin on intestinal ESBL-E carriage detected by rectal swab [9]. Although there was no significant difference in the detection of ESBL-E by rectal swab 28 ± 7 days after the end of treatment, there was lower rectal ESBL-E carriage during treatment and shortly afterwards. We therefore hypothesized that a decolonization regimen with oral antibiotics followed by a recolonization approach that restores the intestinal microbiota for competition with ESBL-E and CPE could be promising. Faecal microbiota transplantation (FMT) has been suggested for this purpose and some animal studies have shown encouraging findings [10–12].

Faecal microbiota transplantation is now established as a safe and effective therapy for patients with recurrent *Clostridium difficile* infection [13,14]. For ESBL-E/CPE decolonization in humans, the scientific literature remains limited to case reports and small, uncontrolled cohort studies [15–19]. In this trial we aimed to combine suppression of carriage through oral antibiotics with FMT using the methodological rigour of a randomized trial design.

Methods

Design and setting, participants, and setting

This was an international, publicly funded, investigator-initiated, randomized superiority trial conducted between February 2016 and November 2017. The study was conducted in four academic centres in Switzerland, France, the Netherlands and Israel (see Supplementary material, Table S1).

Participants

Individuals ≥18 years able to provide informed consent were eligible if colonized with ESBL-E and/or CPE (stool culture at baseline). Patients only colonized with ESBL-E had to have experienced at least one episode of symptomatic infection with ESBL-E requiring systemic antibiotic therapy within ≤180 days before inclusion. Patients with severe immunodeficiency, recurrent aspirations, and intestinal colonization with colistin-resistant strains were excluded (see Supplementary material, Table S2).

Intervention

Patients randomized to the control group were assigned to no specific intervention but were followed up with stool cultures (Fig. 1). Patients randomized to the intervention group were assigned to oral treatment with colistin sulphate (2 million international units 4×/day; CNP Pharma, Fürstenzell, Germany) and neomycin sulphate tablets (350 mg of neomycin base 4×/day; X-GEN Pharmaceuticals, Inc., Horseheads, NY, USA) for 5 days followed by FMT without prior bowel lavage after a pause of one calendar day either using capsules or through a nasogastric application as outlined below (see Supplementary material, Table S3, for the rationale regarding the dose and duration of the oral antibiotics). Two centres (Geneva and Paris) decided to use capsulized FMT instead as it was felt that this would facilitate patient recruitment. The other two centres decided to use the nasogastric approach as planned in the initial protocol because of logistic and administrative hurdles with implementing capsulized FMT.

FMT donor selection and screening

Donor selection criteria were adapted from the 2014 French guidance document for use of FMT in clinical trials (https://ansm.sante.fr/var/ansm_site/storage/original/application/5e5e01018303790194275ded0e02353c.pdf). Donors tested positive for intestinal carriage of multidrug-resistant organisms (MDRO) were excluded (see Supplementary material, Table S4).

The Geneva University Hospitals, Assistance publique – Hôpitaux de Paris and University Medical Centre Utrecht recruited unrelated healthy donors without evidence of or self-reported risk factors for transmittable diseases while the Tel-Aviv Medical Centre used the locally available donor stool bank—see Supplementary material (Fig. S1) for the process for donors. Faecal material was collected with a special stool collection device (Fecotainer®, AT Medical B.V., Enschede, the Netherlands) during the visit. Faecal material was processed in the laboratory under ambient air within 2 h of collection.

FMT preparation and storage

Details of the protocol used for FMT preparation are provided in the Supplementary material (Table S5). The procedure for nasogastric administration was based on the publications by Youngster et al. and Hamilton et al. [20,21]. Aliquots of 80 ml (derived from about 40 g of stool) were prepared for administration to patients in a single session (patients also received omeprazole 20 mg per os on the evening before and on the morning of the day of FMT administration). The protocol for capsulized FMT was based on a procedure developed by the same group as the protocol for FMT using nasogastric application [22]. A higher percentage of glycerol (80% instead of 10% glycerol) was used to increase the stability of the capsules. The dose administered to patients was 15 capsules on two consecutive days (derived from a total of about 15–30 g of faecal material). Capsules prepared according to this modified protocol have been used successfully to treat patients with recurrent *Clostridium difficile* infection in one centre [23]. The FMT preparations

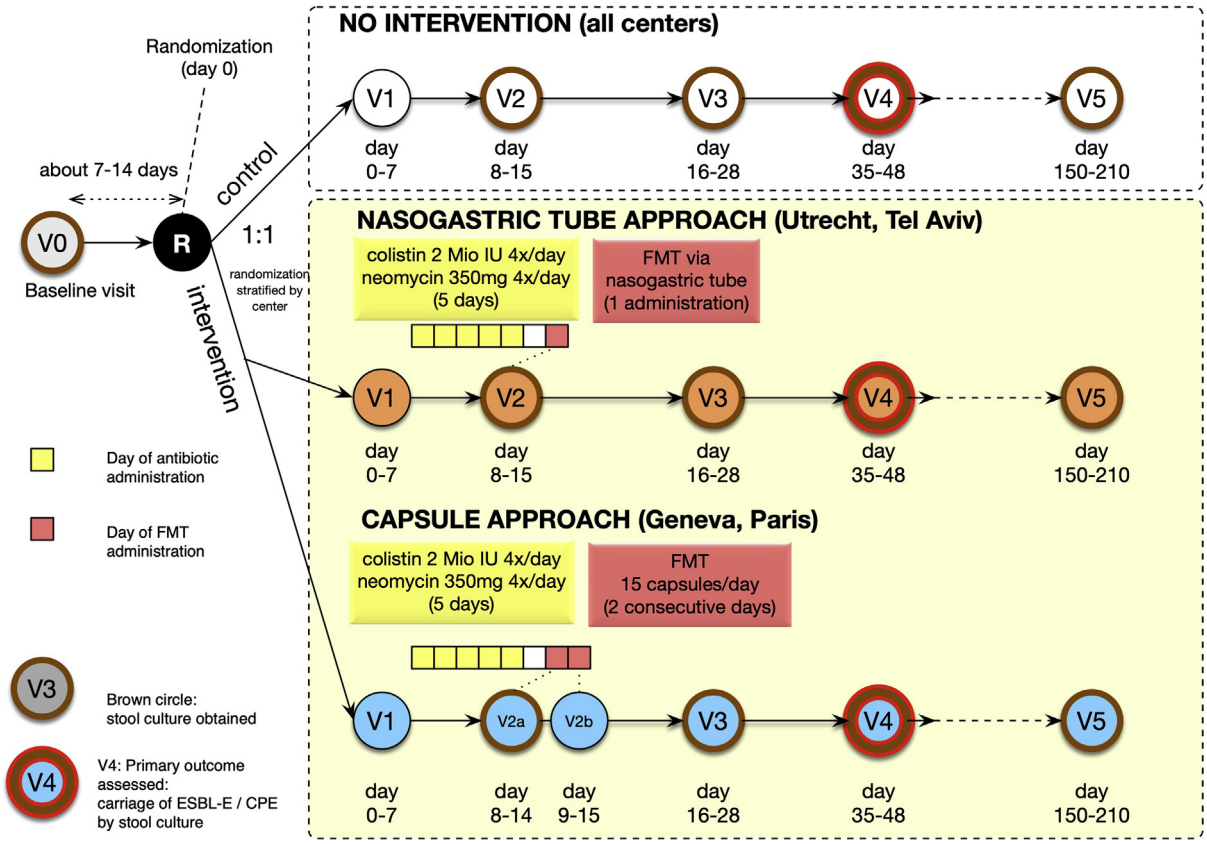

Fig. 1. Study design.

were frozen immediately after preparation at −80°C pending use (within a maximum of 6 months).

Randomization and masking

Separate randomization by centres was generated by a collaborator not involved in patient recruitment or analysis using randomly permuted sequences of different block sizes using an internet-based randomization plan generator (randomization.com). Patients were randomized 1: 1 by phone using sequentially numbered, opaque, sealed envelopes stored in Geneva. This study was not blinded due to the practical barriers to masking the intervention, the disease studied (the risk of auto-administering MDRO was deemed to outweigh the benefits of blinding) and the high degree of objectivity of the primary outcome.

Microbiological methods for detection of ESBL-E and CPE in patients

Stool samples were collected for the following visits: at baseline (V0), 8–14 days after randomization (V2) (after the antibiotics but before FMT in the intervention group), 15–28 days after randomization (V3), 35–48 days after randomization (V4), and 5–7 months after randomization (V5). Stools were collected using a special collection device (Fecotainer®) with a maximum of 72 h (at room temperature) between stool emission and arrival at the laboratory. In the laboratory stool was homogenized with a stomacher and about 100 mg of stool was diluted 1/10 in 1 ml of brain–heart infusion broth supplemented with 20% glycerol. One hundred microlitres of this solution were plated on two different media (CHROMagar ESBL® Chromagar, Paris, France and a second medium based on local availability) and incubated for 24 h at 37°C. Bacterial identification of colonies with distinct morphotypes was based on the colour of the colonies with subsequent confirmation by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). Production of ESBL was confirmed by the double-disc synergy test for *Escherichia coli* and *Klebsiella pneumoniae*. For other *Enterobacteriaceae* ESBL production testing was performed by PCR +/− sequencing based on local protocols. The algorithm for detection of CPE is described in the Supplementary material (Fig. 2). Testing for colistin resistance was performed using E-tests.

Primary outcome

The primary outcome was detectable intestinal carriage of ESBL-E/CPE by stool culture 35–48 days after randomization (V4). Secondary outcomes were safety and tolerability and susceptibility to colistin of ESBL-E and CPE over time.

Patient diary

Patients were asked to fill out a patient diary (see Supplementary material, Appendix S2) for 16 consecutive days.

Sample size

We assumed a spontaneous loss of detectable ESBL-E/CPE carriage in 20% of the patients at 35–48 days after randomization (based on our previous study) in the control group with a further absolute reduction by 40 percentage points in the intervention group [9]. Assuming 80% power at a two-sided significance level of

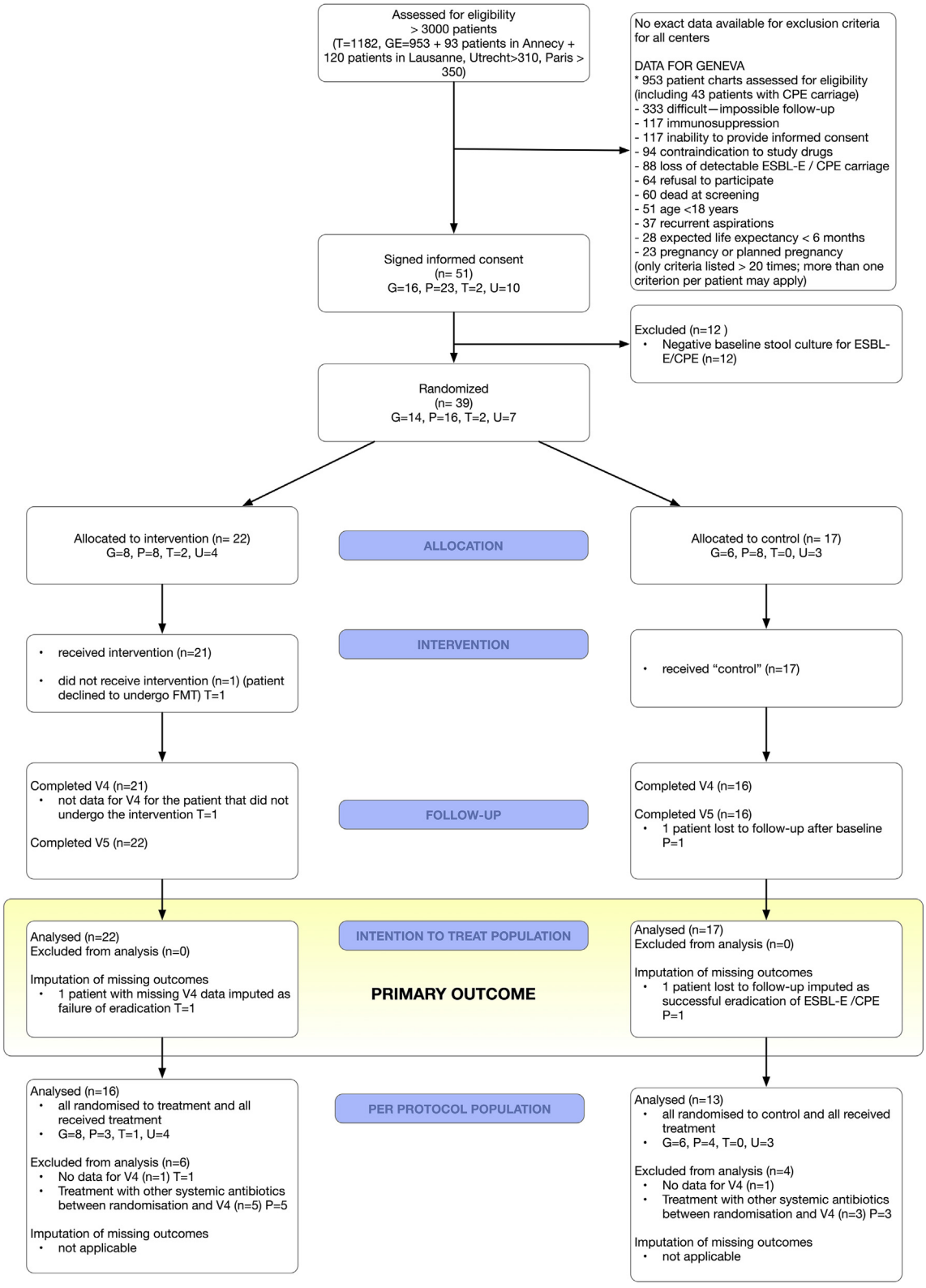

Fig. 2. Study flow chart; G, Geneva; P, Paris; T, Tel Aviv; U, Utrecht. The protocol had originally foreseen multiple imputations for the primary outcome; this idea was, however, abandoned given that only one patient in each group had missing data for the primary outcome. Neighbouring hospitals were also contacted by these centres to increase the pool of potentially eligible patients.

5% and an expected 6% rate of loss to follow up, a targeted sample size of 64 participants was calculated.

Statistical analysis

All data were analysed in STATA 15 (StataCorp, College Station, TX, USA). For the primary outcome the main analysis was performed according to the intention-to-treat (ITT) principle. The ITT population consisted of all randomized patients. Missing data for the primary outcome were imputed according to a 'worst case' scenario (i.e. counted as failure in the intervention group and successful eradication in the control group). Stool cultures were accepted as 'non-missing' if obtained outside the predefined timeframe for the visits, as long as V2 was performed after antibiotic treatment but before FMT in the intervention arm.

We also performed a per protocol analysis (defined after the end of the study but before analysis) where patients were analysed according to treatment received. The per protocol population consisted of patients that fulfilled the following criteria: (i) patients underwent randomization; (ii) patients had non-missing primary outcome data; (iii) patients did not receive non-study systemic antibiotics between randomization and V4. Furthermore, we performed a sensitivity analysis requiring two consecutive negative stool cultures after end of treatment (i.e. either V3/4 or V4/V5) for success. Univariate logistic regression with group assignment as predictor variable and decolonization as outcome variable was performed to calculate OR and 95% CI. Fisher's exact test was used for categorical variables.

Ethics

The study was approved by the institutional review boards and the respective national regulatory agencies at all centres (see Supplementary material, Table S6). All patients provided written informed consent before inclusion.

Results

Patient and donor characteristics

Thirty-nine patients were randomized between February 2016 and June 2017 (G = 14; P = 16; U = 7; T = 2). The study therefore failed to achieve the targeted sample size of 64 patients, mainly because of a delay in starting patient recruitment for various logistical reasons outlined in the Supplementary material (Table S7) and the end of the official project funding period in March 2017. The baseline characteristics of the patients and the study flow chart are illustrated in Table 1 and Fig. 2. Overall, 11 (28%) patients (five control and six intervention) were colonized with CPE. For this study, seven donors were used for stool donations, with donations being used for between one and five patients (see Supplementary material, Table S8).

Withdrawal, loss to follow-up and missing data

One patient in Paris, randomized to the control group, was lost to follow up immediately after randomization. For the ITT, the data

Table 1
Baseline characteristics of study participants

	Control (n = 17)	Intervention antibiotics & FMT (n = 22)	Overall (n = 39)
Female, n (%)	9 (53%)	12 (55%)	21 (54%)
Age (years), median (IQR) range	64 (56–72) range 52–78	70 (57–72) range 23–89	65 (57–72) range 23–89
Body mass index (kg/m^2), median (IQR)	27.4 (25.0–33.2)	23.95 (21.4–29.7)	26.6 (22.3–30.5)
Co-morbidities, n (%)			
Diabetes	4 (24%)	3 (14%)	7 (18%)
Metastatic solid tumour	1 (6%)	0	1 (3%)
Chronic obstructive pulmonary disease	1 (6%)	2 (10%)	3 (8%)
Hypertension	8 (47%)	9 (43%)	17 (45%)
Congestive heart failure	0	1 (5%)	1 (3%)
Severe renal disease	2 (12%)	3 (14%)	5 (13%)
Outpatient status at baseline visit, n (%)	8 (47%)	13 (59%)	21 (54%)
Duration of known ESBL-E/CPE carriage before baseline visit in days median (IQR)	112[a] (25–332)	147 (64–377)	140.5 (56–377)
Number of anamnestic infections with ESBL-E before baseline, median (IQR), range[b]	2 (IQR 1–4) range 1–10	1 (IQR 1–2) range 1–5	1.5 (1–3) range 1–10
History of urinary tract infection with ESBL-E and/or CPE, n (%)	10 (59%)	14[c] (67%)	24 (63%)
Baseline colonization by species, n (%) [n ESBL-E/n CPE][d]			
Escherichia coli	13 (76%) [10/4]	17 (77%) [13/5]	30 (77%) [23/9]
Klebsiella pneumoniae	6 (35%) [5/1]	8 (36%) [7/1]	14 (36%) [12/2]
Enterobacter cloacae	0	1 (5%) [1/0]	1 (3%) [1/0]
Citrobacter freundii	0	1 (5%) [0/1]	1 (3%) [0/1]
Baseline colonization by carbapenemase type, n (%)	5 (29%)	6 (27%)[e]	11 (28%)
OXA	3	4	7
NDM	2	3	5
Baseline colonization with ESBL-E and CPE (different strains), n (%)	3 (18%)	5 (23%)	8 (21%)
Baseline colistin minimal inhibitory concentration (mg/L), median (IQR)[f]	0.25 (0.064–0.5)	0.1575 (0.094–0.5)	0.19 (0.094–0.5)

Abbreviations: CPE, carbapenamase-producing *Enterobacteriaceae*; ESBL-E, extended spectrum β-lactamase *Enterobacteriaceae*; IQR, interquartile range; NDM, New Delhi metallo β-lactamase; OXA, oxacillinase.

[a] One observation missing for one patient.
[b] Data collected only for patients with ESBL-E colonization at baseline; missing data for one patient in the control group and three in the intervention group.
[c] Data missing for one patient in the intervention group.
[d] Some patients colonized with both ESBL-E and CPE.
[e] One patient colonized with both *E. coli* NDM and *C. freundii* NDM.
[f] Highest MIC if several strains present.

for the primary outcome were imputed as negative stool culture at V4. All other patients were followed up until V5; however, one patient from Tel Aviv, assigned to the intervention group but having refused to undergo the intervention, had no stool culture performed at V3 and V4, the result of the latter was imputed as positive.

Treatment adherence

Of the 22 patients in the group assigned to antibiotics and FMT, one patient in Israel did not undergo any part of the treatment and three patients remained under the predefined cut-off of 80% for adherence (i.e. capsules taken) to colistin/neomycin because of diarrhoea. In total, 21/22 underwent FMT (30/30 capsules for all patients receiving capsulized FMT); FMT was administered after a median of 133 days of frozen storage (interquartile range 74–166 days).

Decolonization

According to the ITT analysis 9/22 (41%) patients assigned to the intervention arm were negative for ESBL-E/CPE at V4 whereas in the control arm 5/17 (29%) patients were negative (OR for decolonization success of 1.7; 95% CI 0.4–6.4). Using the more stringent criteria for decolonization, 8/21 (38%) in the intervention group and 4/16 (25%) in the control group reached this outcome (OR 1.8; 95% CI 0.4–7.7). According to the per protocol analysis 8/16 (50%) patients having received the intervention and 3/13 (23%) patients in the control group achieved decolonization (OR 3.3, 95% 0.7–16.8). Fig. 3 (and see Supplementary material, Table S9 and Fig. S3) illustrates the evolution of CPE and ESBL-E carriage over time. The median time from stool emission to arrival at the laboratory was 24 h or less for the 22 patients with available data.

Safety and tolerability

Among the 21 patients in the intervention group having received at least one dose of a study drug, 19 (90%) experienced at least one adverse event (AE) (overall, 104 AE). Of the four severe AE only one was possibly related to the study drugs (hepatic encephalopathy in a patient with liver cirrhosis; see Supplementary material, Table S10). In the 17 patients not having received treatment with follow-up data, 13 (76%) experienced at least one AE (overall 66 AE) and there were two severe AE. The completed patient diary was obtained from 35 patients. Three of 15 (20%) patients in the control group experienced diarrhoea during the 16-day diary period whereas this was the case for 12/21 (57%) in the intervention group (see Supplementary material, Fig. S4). Overall eight patients (five intervention, three control) were treated with antibiotics between randomization and the date of the stool culture for V4 (see Supplementary material, Table S11).

Colistin MICs

No colistin-resistant strains of ESBL-E or CPE were isolated during follow up in the intervention group, but there was one colistin-resistant OXA-producing *E. coli* during follow up in one patient in the control group (Fig. 4).

Discussion

In this international multicentre randomized controlled trial, oral non-absorbable antibiotics followed by FMT resulted in a lower proportion of intestinal colonization with ESBL-E/CPE during follow up compared with control. The strength of the finding is, however, hampered by the failure to achieve the planned sample size, precluding any firm conclusions. Overall, treatment was well tolerated, but the oral antibiotics were frequently associated with diarrhoea, a known side effect of these drugs.

The publication of the trial by van Nood and colleagues in the *New England Journal of Medicine* in 2013 reporting the outstanding efficacy of FMT for the treatment of recurrent *Clostridium difficile* infection has resulted in numerous reports examining FMT for various diseases [24–27]. Because of the largely disappointing results of 'conventional' decolonization regimens for CPE and ESBL-E, there have been a number of case reports and uncontrolled studies examining FMT for the decolonization of intestinal colonization with MDRO. Comparison among these studies is made difficult by

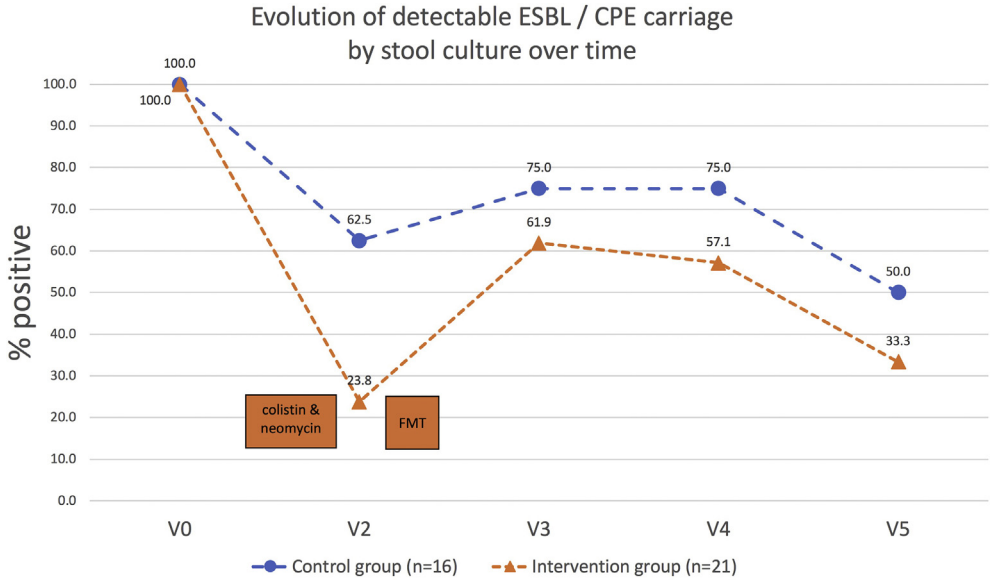

Fig. 3. Evolution of extended spectrum β-lactamase *Enterobacteriaceae* (ESBL-E) and carbapenemase-producing *Enterobacteriaceae* (CPE) carriage over time; only patients with data for all planned stool cultures ($n = 37$) by treatment received (= treatment allocation for this population). Numbers of patients with detectable ESBL-E/CPE carriage by stool culture: for Control group ($n = 16$): V0, 16/16; V2, 10/16; V3, 12/16; V4, 12/16; V5 8/16; and for Intervention group ($n = 21$): V0, 21/21; V2, 5/21; V3, 13/21; V4, 12/21; V5, 7/21.

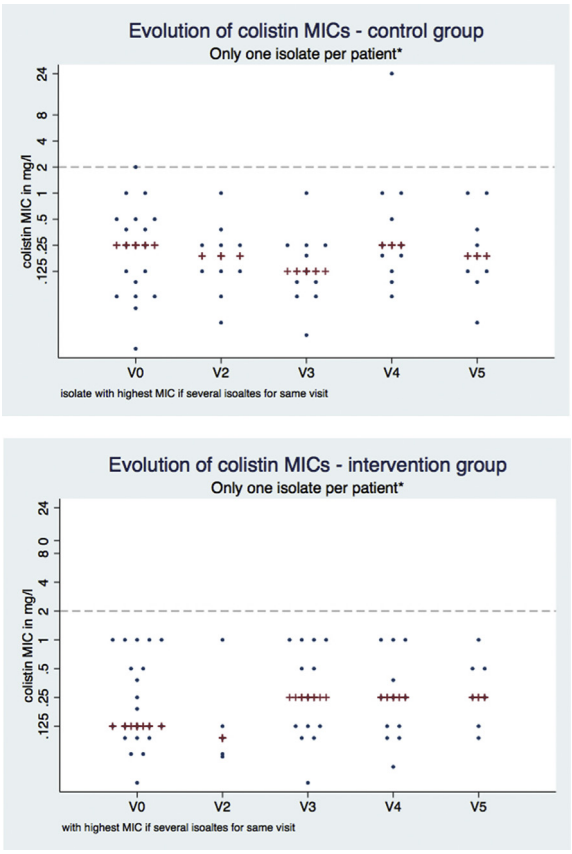

Fig. 4. Colistin MIC assessment, stratified by study arm. The red dashed lined indicates the EUCAST cut-off for colistin resistance in *Enterobacteriaceae* (2 mg/L); note: the *y*-axis is a logarithmic scale.

the inclusion of different patient populations and MDRO, different microbiological techniques and different definitions of decolonization.

A recently published uncontrolled cohort study in France examined FMT for decolonization in eight carriers of carbapenem-resistant *Enterobacteriaceae* and nine carriers of vancomycin-resistant enterococci [18]. One week after FMT, 3/8 with carbapenem-resistant *Enterobacteriaceae* and 3/9 with vancomycin-resistant *Enterobacteriaceae* had negative rectal swabs. A Dutch uncontrolled cohort study examining FMT for decolonization of ESBL-E carriage in 15 patients showed similar results with 3/15 (20%) patients being negative 4 weeks after FMT [15]. A further uncontrolled study from Poland reported successful eradication of intestinal carriage in 15 out of 20 (75%) patients with haematological disease colonized by CPE, ESBL-E or other MDRO 1 month after FMT [19]. Numerous patients also received systemic antibiotics, hampering the interpretability.

Although these studies are based on a sound rationale, it seems essential to use a controlled study design to examine these effects in humans given the known phenomenon of 'spontaneous' loss of carriage of ESBL-E and CPE or decrease below the detection level [7]. Indeed, in our study, 4 of 16 (25%) patients not having received the intervention with available data were negative at V4. The use of high-quality designs seems all the more essential, as many aspects of FMT still remain unknown and the long-term consequences are unclear. In the context of FMT for MDRO decolonization it should be kept in mind that antimicrobial resistance genes can also be acquired through FMT [28]. Furthermore, the exact role of the donor microbiota composition on the impact of FMT on MDRO carriage needs further investigation.

Limitations and strengths

Our study has several strengths, notably the randomized design, the multicentre setting and its long follow up. The major limitation is the failure to achieve the planned sample size for logistical and regulatory reasons (see Supplementary material, Table S7) making it difficult to draw firm conclusions regarding efficacy. Furthermore, most patients in this trial received FMT as capsules, which has been shown to be non-inferior to FMT administered via colonoscopy for the treatment of recurrent *Clostridium difficile* infection, but which administers a smaller amount of FMT with unknown consequences for the likelihood of success for decolonization of MDRO [29]. The fact that we used different techniques of FMT administration introduced heterogeneity into the trial but increased external validity. The use of oral antibiotics before FMT administration is also debatable, given the risk of the emergence of colistin-resistant strains and the difficulty of disentangling the effect of antibiotics from the effect of the FMT.

Conclusion

The results of this trial do not support the routine use of FMT for decolonization. Some other studies examining FMT for MDRO decolonization are currently ongoing (NCT03063437, NCT03167398). Given that the point estimate of the treatment effect for the primary outcome is on the side indicating efficacy it may still be worthwhile to explore the approach in a larger trial using FMT with more faecal material, pooled faecal material from several donors or repeated applications without previous antibiotics or in less selected patient populations (see Supplementary material, Table S12) [30,31].

Previous presentations

Preliminary results of this study were presented at the 28th European Congress of Clinical Microbiology and Infectious Diseases in Madrid, Spain on 23 April 2018 (#O1132) and at the Joint Annual Meeting 2018 of the Swiss Societies for Infectious Diseases, Hospital Hygiene, Tropical Medicine and Parasitology and Tropical and Travel Medicine in Interlaken, Switzerland on 13 September 2018 (#P09).

Transparency declaration

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Trial registration

ClinicalTrials.gov NCT02472600.

Protocol

The full protocol can be obtained from the corresponding author.

Authors contributions

All authors made intellectual input to the manuscript. BDH wrote the study protocol, organized the study in Geneva, coordinated activities among the centres, performed the main study analysis and wrote the first draft of the manuscript. VdL organized the study in Paris and made intellectual input to the study protocol. MW organized the study in Utrecht and made intellectual input to the study protocol. NM was responsible for FMT preparation in Tel Aviv. AM was responsible for FMT preparation in Geneva. NK and AB were responsible for FMT preparation in Paris. TG, VZ and FO co-organized the study in Geneva (patient and donor recruitment and follow up, development of standard operating procedures). XD organized the donor recruitment in Paris. LA organized the microbiological aspects of the study in Paris. YC obtained funding, co-wrote the protocol and supervised the study in Tel Aviv. BF obtained funding, co-wrote the protocol and supervised the study in Paris. MB obtained funding, co-wrote the protocol and supervised the study in Utrecht. SH designed the initial study, obtained funding, co-wrote the study protocol and supervised the study as principal investigator.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2018.12.009.

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